UNIVERSITY OF GONDAR

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES

DEPARTMENT OF MEDICAL MICROBIOLOGY

Prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella Species* among patients with diarrhea attending Gondar town health institutions, Northwest Ethiopia

BY: Tesfaye Andualem (BSc., MSc candidate)

Advisors: Prof. Moges Tiruneh (MSc., PhD, Professor)

Dr. Feleke Moges (MSc., PhD, Associate Professor)

Mr. Dagnachew Muluye (BSc., MSc., lecturer)

A thesis submitted to the Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar for partial fulfillment of the requirements for the degree of Master of Science in Medical Microbiology.

June, 2014

Gondar, Ethiopia

Acknowledgments

I would like to address my deepest gratitude to my advisors Prof Moges Tiruneh, Dr.Feleke Moges, and Mr. Dagnachew Muluye for their timely constructive idea and comments which have been very helpful in improving and guiding the whole research process.

My thanks go to the Ethiopian Public Health Institute (EPI) and Medical Microbiology staff members, especially Mrs. Mesert Assefa and Mrs. Rajiha Abubeker for their willingness and support during serotyping of *Shigella spp.*

I would like to thank also the University of Gondar, College of Medicine and Health sciences, School of Biomedical and Laboratory sciences, Department of Medical Microbiology for giving me the chance to prepare this research paper and sponsoring.

My special thanks to my family for all kinds of assistance and encouragement for the successful completion of this research, especially my brother Asnakew Andualem for his valuable support. Finally I would like to thank Getnet Ayalew for this support during laboratory processing and all the data collectors and study participants who have been involved in this study.

Table of Contents

Acknowledgment i
Table of Contents ii
List of tables iv
List of Figure v
Abbreviations vi
Abstract vii
1. Background and statement of the problem 1
2. Literature review 4
3. Significance of the study 8
4. Objective of the study 9
4..1 General objective 9
4. 2. Specific Objectives 9
5. Materials and Methods 10
5.1 Study area 10
5.2. Study design and period. 10
5.3 Population 10
5.3.1. Source population 10
5.3.2. Study population 10
5.4. Inclusion criteria 10
5.5. Exclusion criteria 10
5.6 Variables 10
5.6.1. Dependent variable 10
5.6.2. Independent variables 11
5.7. Sample size determination and Sampling technique. 11
5.8. Data collection and Laboratory methods 12
5.9. Data Analysis and Interpretation 13
5.10. Ethical Considerations 13
6. Results 14
6.1 Socio -demographic characteristics 14
7. Discussion 21

8. Limitation of the study 23
9. Conclusion 24
10. Recommendation 24
11. References 25
12. Annexes 30

List of tables

Table 1: Socio-demographic characteristics of diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, 2014----------14

Table 2: The frequency of *Shigella species* isolated from diarrheic patients in Gondar town health institutions, Northwest Ethiopia, 2014----------15

Table 3: Clinical characteristics of diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, 2014----------15-16

Table 4: Antimicrobial susceptibility patterns of *Shigella* and *Salmonella* isolates from diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, 2014----------17

Table 5: Multidrug-resistance pattern of *Shigella* and *Salmonella spp.* from diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, 2014----------18-19

List of Figure

Figure 1: Identification of *Shigella* and *Salmonella species* and their antimicrobial susceptibility patterns

Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
CDC	Center for Diseases Control and Prevention
CLSI	Clinical Laboratory Standards Institute
EDHS	Ethiopian Demographic Health Survey
HIV	Human Immunodeficiency Virus
NLF	Non Lactose Fermenter
WHO	World Health Organization

Abstract

Background: Shigellosis and salmonellosis are still global health problems, especially, in developing countries where poor sanitation, lack of clean water supply and proper sewage disposal system exist. The emergence of increased antimicrobial resistance of *Shigella* and *Salmonella* species are global challenges, particularly in developing countries like Ethiopia where increased misuse of antimicrobial agents by human beings occur.

Objectives: To determine the prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella* isolates from patients with diarrhea attending the health institutions in Gondar town, Northwest Ethiopia.

Materials and Methods: Health institutional based cross sectional study was carried out on diarrheic patients attending Gondar town health institutions, February 29, 2014 to May 20, 2014. Systematic random sampling technique was used and stool samples were collected from 372 study subjects. Samples were cultured onto MacConkey and *Salmonella-Shigella* agars and drug susceptibility patterns of the isolates were determined following standard bacteriological method. Data were coded and entered for statistical analysis using SPSS version 20. Data were presented using tables and chi-square and logistic regression was used for explanatory variables.

Result: Of the total of 372 stool cultures, 17(4.57%) *Shigella spp.* and 4(1.08%) *Salmonella spp.* were isolated. Most commonly isolated strains of *Shigella* were *S. flexneri* 11(64.7%) followed by *S. dysenteriae* 3(17.65%), *S. boydii* 2(11.77%) and *S. sonnei* 1(5.88%). *Shigella* isolates presented high resistance rate to ampicillin (94.1%), amoxicillin (88.2), tetracycline (88.2%) and *Salmonella species* were highly resistance to tetracycline (100%), amoxicillin (100%), and ampicillin (75%). However, all isolates of *Shigella* and *Salmonella* were 100%susceptible to ciprofloxacin and norfloxacin.

Conclusion and recommendation: This study revealed that isolates of *Shigella* and *Salmonella* showed high rate of drug resistance to the commonly used antibiotics. However, all the isolates were susceptible to ciprofloxacin, and norfloxacin. Therefore, ciprofloxacin and norfloxacin can be used as drugs of choice for the treatment of *Shigellosis* and *Salmonellosis.*

Key words: Antibiotic susceptibility, prevalence, *Salmonella* and *Shigella*

1. Back ground and statement of the problem

The *Shigellae* are members of the enterobacteriaceae, non lactose fermenters, non motile, and non gas producers gram negative rods (1). There are four species of *Shigell*a, which includes *S. dysenteriae (group A), S. flexneri (gropuB), S. boydii (group C),* and *S. sonnei (group D)* (1). *Shigellae* are transferred from person to person usually by asymptomatic carriers and via contaminated food, flies, faeces, fingers, and water (1). To initiate infection, as few as 100 ingested *Shigella* organisms are enough. After the organisms enter the human body, they remain in the cytoplasm of the epithelial cells and spread laterally to invade adjacent cells which result in the formation of abscesses and ulcerations with high concentration of neutrophils in the stools (1).

Shigellosis is only a human disease caused by the four species of genus *Shigella* and is characterized by increasing in frequency of stool motion and increase in number with the presence of blood, mucous and pus in the stool (1, 2).

Shigellosis is endemic throughout the world where, it is held responsible for some 165 million cases of severe dysentery. The majority of these cases occur in the developing countries. More than one million people are estimated to die from *Shigella* infection each year. Since the late 1960s, pandemic waves of *Shigella* dysentery had striken Sub-Saharan Africa, Central America and East Asia (3).

In Africa, an estimate of 115 people die of diarrheal diseases every hour, mostly of shigellosis and salmonellosis which are linked to contaminated food and water due to poor sanitation and hygiene (4). In Ethiopia, one in every 17 Ethiopian children dies before the first birthday and one in every 11 children dies before the fifth birthday. According to the Ethiopian demographic and health survey (EDHS) report of 2011, 13 percent of the children under age five were reported to have had diarrhea, and 3% had diarrhea with blood in to the two-week periods before the survey (4). It is highest (18%) among children residing in households that drink water from unprotected wells. The prevalence of diarrhea in children residing in Amhara region was also found to be 13.5% (4).

Genus *Salmonella* are a member of enterobactericeae, non lactose fermenters, motile and gas producer gram negative rods (1). Transmission of these organisms are from person to person via fecally contaminated food, water or through direct faecal oral route (5, 6). They invade the mucosa of the small and large intestines and produce inflammation. Invasion of epithelial cells induces an inflammatory reaction which cause diarrhea due to salmonella (7). Salmonellosis is a disease caused by a large group of bacteria of the genus *Salmonella* (1).

Two hundred million to more than one billion cases of diarrhea result worldwide due *Salmonella* infections every year, leading to 3 million deaths (5).The highest incidence of infection is among the very young and elderly. Mortality is highest in children less than one year old. The increase susceptibility of this age group may be due to the fact that children less than 2 months old produce little hydrochloric acid, a natural barrier to many microorganisms (5).

The wide spread occurrence and distribution of *Salmonella* infection in Ethiopia is increased. It is due to contamination of water, food, and poor sewage disposal system. The very young, elderly, and immunocompromized individuals are particularly more susceptible to *Salmonella* infections at a lower infective dose than healthy adults (8).

Due to different factors in the last few decades, *Shigella* and *Salmonella* have become increasingly resistant to the most commonly used antimicrobials from time to time which results in some challenges in selecting the drug for therapeutic management (9, 10). The emergence of antibiotic resistant *Shigella* and *Salmonella* are serious problems in antimicrobial therapy globally. The incidence varies with the area of isolation of these strains. The progressive increase in antibiotics resistance among these pathogens in developing countries is also becoming a critical area of concern (7).

In developing countries like Ethiopia, resistance may be acquired mostly by selective pressure due to indiscriminate and misuse of antibiotics (11, 12). This leads for the emergence of resistance strains of *Shigella* and *Salmonella* which may be difficult for treatment and prevention (13, 14). In the 1940s, shigellosis was treated successfully with sulfa- drugs. In the 1950s, it was treated with tetracycline (5). In the 1970s ampicilin was the drug of choice for the treatment of bacillary dysentery (15). After the pathogen began to develop resistance to ampicilin the new drug trimethoprime- sulphamethoxazole(TMP-SXT) was used (16). Even if the drug of choice

in that time was TMP-SXT, in the 1980s, the *Shigella* species started to develop resistance to the drug (17). Then the emergence of resistance strains continues with any new drugs.

Until about 1960, nearly all *Salmonellae* were sensitive to a wide range of antimicrobial agents but since 1962 emergence of resistance, frequently plasmid mediated, have appeared in *Salmonella* worldwide. The relative importance of antibiotic resistance, and the serotypes in which it occurs, differs from country to country (5).

Knowing the prevalence and the local patterns of antimicrobial susceptibility of *Shigella* and *Salmonella* species is important for reducing the burden of the diseases(7). Therefore, the aim of this study is to determine the prevalence and susceptibility patterns of *Shigella* and *Salmonella* isolates from patients with diarrhea attending the health institutions in Gondar town, Northwest Ethiopia.

2. Literature review

2.1 Shigellosis

In most cases, shigellosis in developed countries was due to *S.sonnei*, where as shigellosis in developing countries was due to *S.flexneri* followed by *S.dysenteriae* (7, 18, 19).

A report in the United States indicates that an average of 20,000 to 30,000 cases per year were reported. *S.sonnei* accounted nearly 80% of all *Shigella* isolates while *S.dysenteriae* accounted nearly 1% (1). This finding was in line with studies done in Tehran and Trinidad which showed that the major species causing shigellosis was *S. sonnei* (20, 21). However, in Abidjan, Côte D'Ivoire the predominant species were *S. flexneri* followed by *S. boydii*, *S.sonnei*, *S. dysenteriae* (22).

A study conducted in Iran, on children who had diarrhea, indicated that the prevalence of shigellosis was high (14.5%) whereas, a community based study done in southern Trinidad showed the prevalence of shigellosis was relatively low (8%) (20, 22).

The frequency of isolation, distribution and prevalence of *Shigella* species varies from time to time and from region to region (23). In Iran, the most frequently isolated species was *S.sonnei* followed by *S. flexneri, S.boydii,* and *S. dysenteriae* (21) which was in line with a report in Trinidad (20) and in Ethiopia, *S.flexneri* was the most frequent followed by *S.dysemnteriae, S.boydii*, and *S. sonnei (13, 24).* In a study done in Indonesia, the prevalence of shigellosis on children having diarrhea, showed 9.3% (25) whereas study conducted in Côte D'Ivoire showed that the prevalence rate of *Shigella* among diarrheal patients was 2.9% (22) and in Ethiopia, Gondar (7.5%) (24).

A hospital based study conducted in Tanzania on patients with bloody diarrhea, the *Shigella* species isolated were 69(14%). Of the 69 *Shigella* species isolated, 62(90%) were *S.flexneri*, 7(10%) were *S.dysenteriae,* and none of them were found to be *S.boydii* and *S. sonnei (19)* whereas a surveillance study done in Kenya indicated that 16%(549/3445) *were positive (26). Moreover, another study done in Kenya showed that the prevalence of Shigella was 43.7%* (27).

Cross sectional study conducted in Southwest Ethiopia, Jimma on pediatric out patients having diarrhea 77/384 (20.1%) were positive, in which *S.flexneri* was the most common followed by

S.dysenteriae and *S.boydii*. Of these isolates, more than 53% were resistance to tetracycline, ampicilin, cephalotin and 25-40% were resistance to trimethoprim-sulphamethoxazole, carbencillin(28) whereas a health institutional based study conducted in Ethiopia, Hawassa on diarrheal patients showed,100/ 289 (34.6%) were positive for shigellosis and 99(99%) were *S.flexneri* and 1(1%) were *S.dysenteriae* and all the isolates were susceptible to nalidixic acid and gentamycin (29).

A study conducted in Ethiopia, Hawasa, on stools of children showed the rate of isolation for *Shigella* was 7 % (30) and another cross sectional study done in Ethiopia, Hawassa, on stools of food handlers in catering establishments at Hawassa University showed the prevalence of shigellosis was 0.4% (31) whereas in Jimma on the same study group the prevalence of *Shigella* was 2.3% (32) and in Harar it was 6.7% (33).

Of the 76 isolates of *Shigella* from Ethiopia Addis Ababa, *S.flexneri* was the most frequent(54%) followed by *S.dysenteriae* (22.4%), *S.sonnei* (15.8%), and *S.boydii* (7.8%) (13), while another study done in Ethiopia, Gondar, showed that the prevalence of *Shigella* were (7.5%) (24) and (3.1%) (34). Among the 90 isolates of *Shigella* the most common species were *S.flexneri* (72.2%) followed by *S.dysenteriae* (10%), *S.boydii* (8.9%), and *S.sonnei* (8.9%) (34).

Another cross sectional study conducted in Ethiopia, Gondar Hospital showed that the prevalence of *Shigella* was 15.6% (12) and a retrospective study done on the same area also reported as 7.4% (35). Moreover, a cross sectional study on the same area in HIV infected and uninfected patients from 2003 -2004 indicated that of the 391 stool samples (8.7%) were positive (36) which was different from a study on this area on diarrheal patients(16.9%) on 384 stool specimens positive for shigellosis (34).

2.2 Salmonellosis

Salmonella species are leading causes of acute gastroenteritis in different countries, especially in the developing countries like Ethiopia where substandard hygienic conditions and unsafe water supplies are common (37).

The World Health Organizations estimated 16 million new cases of salmonellosis and an estimated of 600,000 deaths of typhoid fever were reported (38).

A hospital based study in china on diarrheal patients showed that the prevalence was 3.58%, of 8405 diarrheal cases, 301 were positive and *S.thyphemerium* and *S.enrerica* were commonly isolated (39).

The prevalence rates of salmonellosis were varies from the age groups. A study which was done in Brazil on under five children showed that the pathogen were found more: other persons who were carriers and who were taking care of them may have transmitted to them by hands and foods (40). However, a study done in Nigeria indicates that the prevalence of salmonellosis increased as age increased (41).

A hospital based study done in Tanzania on the stools of bloody diarrhea showed that the prevalence of *Salmonella* was 0.8%(19) whereas a study done in Kenya showed the prevalence of *Salmonella* was 3.3% (27).

A cross sectional study conducted in Ethiopia, Jimma reported the prevalence of *Salmonella* as 15.4% (42) and another study done in the same area jimma showed that the prevalence of *Salmonella* was 6.2% (32) whereas study conducted in Ethiopia, Hawasa on children with diarrhea showed that the prevalence of *Salmonella* was 2.5%(30).

In a study conducted in Ethiopia ,Harar, the prevalence of *Salmonella* was 11.5%(33) whereas in another cross sectional study done in Ethiopia ,Hawasa, on food handlers of catering Hawassa University showed no *Salmonella* species was isolated (31). However, another study which was conducted in Gondar, Ethiopia showed 1.6% (12).

2.3. Antimicrobials susceptibility patterns of *Shigella* and *Salmonella*

Shigella and *Salmonella* species were resistance to the commonly used drugs such as tetracycline, amoxicillin, ampicilin, cotrimoxazole, trimethoprime-sulphamethoxazole whereas they were frequently sensitive to naldixic acid, norfloxacin, ciprofloxacin, gentamycin (7, 13, 24, 29). However, study done in Hawasa showed that *Salmonella spp*. was highly sensitive to the commonly used antibiotics: ampicilin (100%), tetracycline (100%), chloramphenicol (100), cotrimozaxxole (100%), amoxacilin (100%), and gentamycin (100%) (30).

A cross sectional study which was done in Eastern Ethiopia, Harar on diarrheal patients showed that the sensitivity of the *Salmonella* isolates were 0.0% to ampicillin; 0.0% to amoxicillin; 14.2% to tetracycline; 28.6% to chloramphenicol; 89.3% to norfloxacin; and 92.8% to gentamicin and *Shigella* had sensitivities of 0.0% to ampicillin; 0.0% to amoxicillin; 11.8% to tetracycline; 41.2% to chloramphenicol; 88.2% to norfloxacin; and 94.1% to gentamicin (33) which was in line with study done in Ethiopia, Gondar showed that the continued sensitivity of *Shigella* to gentamycin, and ciprofloxacin and their wide spread resistance to tetracycline, ampicilin, cotrimoxazole, and chloramphinicol (35).

Study conducted in Ethiopia, Gondar on Serodiversity and antimicrobial resistance patterns of *Shigella* isolates showed that all the isolates had highest resistance rates to tetracycline (90%), cotrimoxazole (84.5%), ampicilin (78.9%), chloramphinicol (67.8), and low resistance rate to gentamycin (12.2), ciprofloxacin (2.2%), and norfloxacin (1.1%). All the isolates was sensitive to naldixic acid and ceftriaxone(24). Moreover, study on the same area showed the highest resistance to Tetracycline (87.7%), Ampicillin (81.5%), Co-trimoxazole (75.4%), Chloramphenicol (50.8%), and low resistance rate to Gentamycin (10.7%) , and Ciprofloxacin (9.2%) (7).

3. Significance of the study

Diarrheal diseases are the main health problems in developing countries. In Ethiopia, diarrheal diseases which include shigellosis and salmonellosis are said to be common and are difficult to manage because of the emergence of drug resistance strains of the agents. About half of the *Shigella* and *Salmonella* isolates obtained from Ethiopian patients with diarrhea had shown multi drug resistance patterns to the commonly used antibiotics. These finding indicates that our communities suffer from drug resistance shigellosis and salmonellosis. Therefore, the present study may address the distribution of *Shigella* and *Salmonella* and their patterns of antimicrobial susceptibility which may have a paramount importance for researchers, policy makers, local , regional, and national health administrative and awareness of professionals to look for a possible means of control and preventive majors.

4. Objective of the study

General objective

To assess the prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella* species among patients with diarrhea attending Gondar town health institutions, Northwest Ethiopia.

Specific Objectives

- To determine the prevalence of *Shigella* among patients with diarrhea attending health intuitions in Gondar town.
- To determine the prevalence of *Salmonella* species among patients with diarrhea attending health intuitions in Gondar town.
- To determine antibiotic susceptibility patterns of *Shigella* and *Salmonella* isolates.

5. Materials and Methods

5.1 Study area

The study was conducted in Gondar town health institutions. Gondar is a capital city of North Gondar administration zone, in Amhara region, Northwest Ethiopia. The town is located at 748 km r from Addis Ababa, the capital city of Ethiopia and 180 km from Bahir dar. The zone has an estimated total population of 3,245,577, with a male to female ratio is 1:1 and has 34 woredas and 3 Hospitals. Specifically the town has an estimate of > 300,000 total populations (43) with one referral hospital (University of Gondar Referral Hospital) and 8 health centers that include Woleka, Gondar, Gebrieal, Ginbot 20, Maraki, Azezo, Tseda, and Blajig Health Centers which are currently giving health service to the community.

5.2. Study design and period

Health institutional based cross- sectional study was conducted from January 2014 to May 2014.

5.3 Populations

5.3.1. Source population

All the populations who were living in and around Gondar town were the source of population.

5.3.2. Study population

The study populations were patients having diarrhea attending health institutions in Gondar town during the study period.

5.4. Inclusion criteria

- All patients with diarrhea.

5.5. Exclusion criteria

- All patients with diarrhea who had taken antibiotics treatment in the last 14 days and /or all patients who were not lived in Gondar for the last one week.

5.6 Variables

5.6.1. Dependent variable

- Prevalence of *Shigella* and *Salmonella* species
- Antimicrobial susceptibility patterns of *Shigella* and *Salmonella* species

5.6.2. Independent variables

The independent variables of the study are:

age, sex, residence, occupation, hygienic standard, availability of toilet, type of water source used, accessibility of health institutions

5.7. Sample size determination and Sampling technique.

Taking prevalence from the previous study which was conducted in Gondar town, Ethiopia (16.9%) on diarrheal patients (7), Using 95 % of confidence interval with 4% of margin of error sample size was calculated as follows.

$$N=\frac{Z2\ P(1-p)}{W^2}$$

Where:

n = No of sample that will be included

$\frac{Z2}{2}$ = confidence level

P= prevalence from the previous study.

W= acceptable difference

$$n= \frac{(1.96)2\ x\ 0.169(1-.169)}{(.04)2}$$

n= 338

Adding the 10 % contingency 372

So, the sample size was 372

Systematic random sampling technique were used.

In the study area all the patients who were attended in one month before study was 539. The estimated number of patients in the data collection time (February 29 -May 20) was 1437.

To determine the k value. Population size (N) =1437, sample size=372

k=N/n=1437/372=3.86, K=4

Then every 4^{th} cases of diarrheal patients were selected as study subjects.

5.8. Data collection and Laboratory methods

Socio-demographic characteristics was assessed and recorded from the study participants by pretested structured questionnaire.

Sample collection and processing

Stool samples from diarrheic patients were collected in a clean, dry, disinfectant-free suitable wide-necked container and immediately transferred to Selenite F broth as a transport medium and inoculate onto *Salmonella-Shigella* agar (Oxoid) and MacConkey (Oxoid), and incubated at 37^0C for 24 hours. After incubation, the plates were examined for growth and gram stain was done and further bacterial species were identified following standard biochemical test procedure. Biochemical tests performed were triple sugar iron agar, indole, urea, Simon's citrate agar, lysine iron agar, and motility tests. *Shigella species* were serogrouped by the slide agglutinations test using commercially available polyvalent O antisera for *S. dysenteriae*, S. *flexneri, S. boydii* and *S. Sonnei* (38).

Antimirobial Susceptibility test

SSuspension of test organisms were prepared by picking pure colonies with a sterile wire loop suspended in sterile nutrient broth and incubated for 2 hrs (38). The density of suspensions to be inoculated were determined by comparing with 0.5 McFarland standards. A sterile cotton swab was used and the excess suspension was removed by gentle rotation of the swab against the surface of the tube and then spread evenly over the Muller Hinton agar plate. Susceptibility testing was performed on isolates using agar disc diffusion technique against ampicillin (10 µg), amoxicillin (10µg), tetracycline (30 µg), trimethoprime-sulphamethoxazole (30µg), gentamycin (10µg), kanamycin (25µg), nalidixic acid (30 µg), chloramphenicol (30µg), norfloxacin(10µg), ciprofloxacin (5µg)) and cefaclor (30 µg). he plates will be left at room temperature for 30 minutes for diffusion then incubated for 18-24 hours at 37^0C. After 18-24 hrs, the zone of growth of inhibition around each disc was measured in millimeters, using a metal caliper, and interpreted as sensitive; intermediate and resistance following the method of CLSI(38, 44).

Data Quality control

The reliability of the study findings was guaranteed by implementing quality control (QC) measures through the whole process of the laboratory work. All materials, equipment, and procedures were adequately controlled. Culture media was tested for sterility and performance. Pre analytical, analytical, and post analytical stage of quality assurance those were incorporated in standard operating procedures (SOPs) of the Microbiology Laboratory at the University of Gondar was strictly followed. Standard reference strains of *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used to chech the performance of culture medias, biochemical tests and antimicrobial discs (38, 44).

5.9. Data Analysis and Interpretation

Data were checked for completeness and all responses to the questionnaire were coded against the original English version and analysis was made by using SPSS version 20 software.Data were analyzed by using tables and chi-square. Logistic regression was used for explanatory variables. P-values ≤ 0.05 were considered as statistically significant.

5.10. Ethical Considerations

Ethical clearance was obtained from research and ethics committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Permissions were obtained from the concerned bodies of the Zonal Health office and selected health institutions. Informed consent was obtained from each study participants. For each confirmed cases, the responsible clinician of the patient was informed and treatment was started as per the guideline. For children assent was obtained from their parents or guardians.

6. Results

6.1 Socio - demographic characteristics

A total of 372 study participants were included in this study, of which 180 (48.4 %) were males and 192 (51.6 %) were females. The ages of the study participants ranged from 1 to 84 years with a mean age of 21.18. Among the study participants 274 (73.7%) lived in urban areas. One hundred two (27.4%) diarrheic patients were from primary school and majority 147 (39.5%) of the diarrheal patients were under 18 years who are not eligible for legal labor working (Table 1)

Table 1: Socio-demographic characteristics of diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May 2014

Characteristics	Number	Per cent
Sex		
Male	180	48.4
Female	192	51.6
Age in (years)		
<20	189	50.8
20-40	139	37.4
41-60	32	8.6
≥61	12	3.2
Educational status		
Under 5 years *	68	18.3
Illiterate	35	9.4
Wright and read	58	15.6
Primary school (1-8)	102	27.4
Secondary school (9-12)	74	19.9
College/University	35	9.4
Occupational status		
Government employees	33	8.9
Private employees	39	10.5
Non employees	99	26.6
Merchant	29	29.8
Farmer	25	6.7
Under 18 years **	147	39.5
Residence		
Urban	274	73.7
Rural	98	26.3

**Under 18 years-they are illegible for work, *under 5 years-they are illegible for school.

6.2 Prevalence of *Shigella* and *Salmonella species*

Of the 372 study participants with diarrheal diseases 17(4.57%) *Shigella spp.* and 4(1.08%) were *Salmonella spp* were isolated. Of the *Shigella spp* isolates, 11 (64.7%) were *S. flexneri*, the predominant isolates followed by *S. dysenteriae* 3(17.65%), *S. boydii* 2(11.77%) and *S. sonnei* 1(5.88%) (Table 2).

Table 2. The frequency of *Shigella* and *Salmonella species* isolated from diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May 2014

Bacterial isolates	**Number (%)**
Shigella speies	**17 ( )**
S.flexneri	11(64.7)
S.dysenteriae	3(17.65)
S.boydii	2(11.77)
S.sonnei	1(5.88)
Salmonella species	4( )
Total	21 (100)

6.2.1 Clinical characteristics of diarrheal patients

Fever, abdominal pain, vomiting, headache were the major clinical symptoms of the patients in this study (Table 3). Of the total participants, abdominal pain was found to be 315(84.7%) of the study subjects

Table 3. Clinical characteristics of diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May 2014

Clinical characteristics	Number	per cent
Abdominal pain		
Yes	315	84.7
No	57	15.3
Vomiting		
Yes	164	44.1
No	208	55.9

Fever		
Yes	233	62.6
No	139	37.4
Headache		
Yes	214	57.5
No	158	42.5
Joint pain		
Yes	161	43.3
No	211	56.7

Of the 17(4.57%) *Shigella* isolates, bloody diarrhea was found in 6(35.32%) and mucoid diarrhea was 7(41.1%) whereas watery diarrhea was 3(17.6%) and 8(47.1%) had previous diarrhea (X^2==0.51, $df = 2$, p value=0.599)

6.2.2 Risk factors for shigellosis and salmonellosis

Majority of the *Shigella spp*. isolated from patients who ate their food in street vendors, other compounds and hotels were 10(58.8%), x (%), respectively, while 7(41.2%) were who had eaten in their home. Most of them did not have liquid waste disposal system 11(64.7%) and they had used non-boiled water 16(94.1%). Thirteen (76.5%) patients did not wash hands before preparing food (X^2=2.15, $df = 1$, p value=0.143) whereas only 5(29.4%) washed their hands after defecation. Age of the patients was not associated with shigellosis (χ^2=0.661, $df = 3$, p-value= 0.882) and sex of the patients had also not associated with shigellosis (X^2=0.777, $df = 1$, p-value=0.378). Bivariate and multivariate logistic regressions were employed but none of the variables were statistically significant with shigellosis.

6.3 . Antimicrobial susceptibility pattern of *Shigella* and *Salmonella* species

The antimicrobial susceptibility patterns of the *Shigella* and *Salmonella* isolates were presented in Table 4. Strains of *Shigella species* were resistant to ampicilin (94.1%), tetracycline (88.2%), amoxicillin (88.2%), but susceptible to norfloxacin (100%), and ciprofloxacin (100%). *Salmonella* isolate were also resistant to ampicilin (75%), amoxicillin (100%) and tetracycline(75%), but highly susceptible to norfloxacin(100%) and ciprofloxacin(100%).

Table 4.Antimicrobial susceptibility patterns of *Shigella* and *Salmonella* isolates diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, 2014.

Bacterial isolates	**Resistance Pattern to antimicrobial agents Number (%)**										
	AMP	**AMOX**	**TTC**	**CN**	**K**	**NA**	**CEF**	**SXT**	**C**	**NOR**	**CIP**
Shigella(n=17)	16(94.1)	15(88.2)	15(88.2)	7(41.2)	7(41.2)	5(29.4)	11(64.7)	10(58.8)	3(17.6)	0(0)	0(0)
Salmonella(n=4)	3(75)	4(100)	3(75)	1(25)	1(25)	1(25)	4(100)	2(50)	0(0)	0(0)	0(0)
total 21	19(84.55)	19(94.1)	18(81.6)	8(33.1)	8(33.1)	6(27.2)	17(82.35)	12(54.4)	3(17.6)	0(0)	0(0)

Key: AMP-Ampicillin, Amox-Amoxacillin, TTC-Tetracycline, CN-Gentamycin, K-kanamycin, NA-Nalidixic acid, CEF-Cefaclor, SXT-Trimethoprime-Sulphamethoxazole, C-Chloramphenicol, NOR-Norfoloxacin, CIP-Ciprofloxacin.

6.3.1 Multiple Antimicrobial resistance pattern of *Shigella* and *Salmonella* isolates

The results of multiple antimicrobial resistant patterns were presented in Table 5. Of the 17(4.57%) of the *Shigella* isolates 16(94.1%) showed multi-drug resistant, while one of them was sensitive to all antimicrobial agents tested. The *Shigella spp*. showed multi drug resistance up to 8 antibiotics and the *Salmonella spp*. showed up to 5 antibiotics

Table 5: Multidrug-resistance pattern of *Shigella* and *Salmonella* isolates from diarrheic patients attending Gondar town health institutions, Northwest Ethiopia, February to May 2014

Bacterial isolates	**Number of antibiotics resisted**	**Antibiotics tested**	**Total (%)**
Shigella species	R4	AMP, AMOX, TTC, SXT	2(12.5%)
		AMP, AMOX, TTC, CEF	2(12.5%)
		AMP, TTC, CF, NA	1(6.25%
	R5	AMP, AMOX, TTC, CN,KAN	1(6.25%
		AMP, AMOX, SXT, CF, NA	1(6.25%
		AMP, AMOX, CF, CN, NA	1(6.25%
	R6	AMP, AMOX, TTC, SXT, CEF, KAN	1(6.25%
		AMP, AMOX, TTC, SXT, CEF, NAL	1(6.25%
		AMP, AMOX, TTC, SXT, CN, KAN	1(6.25%
		AMP, AMOX, TTC, SXT, CEF, CN	1(6.25%
		AMP, AMOX, TTC, CEF, KAN, NAL	1(6.25%
	R7	AMP, AMOX, TTC, SXT, CEF, CN, KAN	2 (12.5%)
	R8	Amp, Amox, TTC, SXT, CEF, CN, K, C	1(6.25%)
Salmonella species	R3	AMOX, CEF, AMP	1(25%)
	R4	AMOX, CEF, AMP, SXT	2(50%)
	R5	AMOX, CEf, AMP, CN, K	1(25%)

Key: AMP-Ampicillin, Amox-Amoxacillin, TTC-Tetracycline, CN-Gentamycin, K-kanamycin, SXT-Trimethoprime-Sulphamethoxazole,C-Chloramphenicol, NA-Nalidixic acid, CEF-Cefaclor NOR-Norfoloxacin, CIP-Ciprofloxacin.

7. Discussion

In this study, the isolation rate (4.57%) of *Shigella spp.* was almost in line with studies from Indonesia (9.3%) (25) and Trinidad (8%) (20). This was lower than studies done in Tehran (14.5%) (21), Kenya:(16%) (26), (43.9%) (27). The low rate of isolation as observed in the present study may be due to differences in the method of sample collection, isolation identification. The rate of *Shigella* (Table 2) was also lower than previous studies in Ethiopia: Jimma (20.1%) (28), Hawasa (34.6%) (29) , and Gondar: (16.9%) (7), (15.6%) (12) but in agreement with Harar (6.7%) (33) and Gondar :7.4%(35), 7.5%(24). However, higher than study from Hawasa (0.4%) (31). This might be due to differences in awareness of the people about personal and environmental hygiene from the continuous health education made by the different health educators in the different health institutions against of shigellosis and salmonellosis.

In the current finding the isolation rate (1.076%) of *Salmonella spp.* was found to be lower than studies done in Ethiopia at different places: Harar (11.5%) (33), Jimma : (15.4%) (42), (6.2%) (32). However, it was consistent with the findings reported in Tanzania (0.8%) (19) and in Ethiopia: Hawasa: (0%) (31), (2.5%) (30), Gondar: (1.6%) (12), and (0%) (34). This might be due to the increasing awareness of the people about personal and environmental hygiene made by the health institutions and other partners.

Our findings showed that *S. flexneri* (64.7%) (Table 2) was the predominant serogroup in the present study which was found to be higher than the finding reported in Addis Ababa (54.0%) (13), and lower than the findings reported in Tanzania (90%) (19), Gondar (72.2%) (24) and in line with a report in Indonesia (63.2%) (25). However, the prevalence of *S .flexneri* at the same study area decreased from (72.2 %) in 2008 (24) to (64.7%) in the present study, whereas the prevalence of *S. dysentriae* increased from (10%) in 2008 (24) to (17.65%) in the present study. The current finding indicated that *S. Sonnei* (5.88 %) (Table 2) were the least frequently isolated spp. which was in line with the previous study conducted in the same study area 8.9%((24).

Majority of *Shigella* isolates were found in mucoid diarrhea 8(47.1%), not washing hands after defecation 12 (70.6%) and not washing hands before preparing food 13 (76.5%). However, it was not stastically significant associated with an increasing of infection with *Shigella* wich was in line with study done on childhood diarrhea in sub-saharan africa (45).

The emergence of antibiotic resistance among *Shigella* and *Salmonella spp. isolates* are serious problems in antimicrobial therapy globally, especially in developing countries. The incidence varies with the area of isolation of these strains (7). When results of the antimicrobial susceptibility patterns of *Shigella* and S*almonella species* in this study compared with earlier reports from other parts of Ethiopia showed that there has been a change in resistance pattern to antibiotics use in Ethiopia. It showed a high degree of resistance to the commonly used antimicrobials. Similar observations were also reported in Indonesia(25), Iran(23), Tehran(21), and Ethiopia(13, 32, 33) (Table 8). According to this study, therefore: ampicillin, tetracycline, amoxicillin and SXT are no longer effective for the treatment of shigellosis and salmonellosis in the study area

More than 58% of *Shigella* isolates were resistance to amoxacillin, tetracycline, ampicillin, cefaclor, and 29-42% of the isolates were resistance to gentamicin, kanamycin, and nalidixic acid. This was much higher than the findings reported in the 1980s, and 1990s(45).The resistance to all other antibiotics in this revealed higher rate of resistance than the findings reported in the 1980s, 1990s and (45). This may be due to overuse of antimicrobials in the area.

In this study, the overall multiple drug resistance pattern of *Shigella* were 16(94.1%) of the isolates (Table 5); this was higher than studies conducted in Ethiopia, Gondar: (87.8%) (35) and (68%) (34) and lower than study done in Jimma (100%) (32).

The present study showed that there was an increasing frequency of resistance of *Shigella* strains to nalidixic acid (58.8%) and gentamicin (41.2%) as compared with the previous reports in Ethiopia: nalidixic acid (2.7%) (13) and gentamicin (0%) (13) and nalidixic acid(0%) (24) and gentamicin (12.2%) (24).

We identified no resistance to chloramphenicol, norfloxacin and ciprofloxacin. Other studies also found that little resistance to chloramphenicol, norfloxacin and /or no resistance to ciprofloxacin at the same study area (24, 34).Thus, at the present time ciprofloxacin and norfloxacin are the best drugs of choice for the treatment of shigellosis and salmonellosis in the study area.

8. Limitation of the study

- Due to unavailability of antisera, serotyping of *Salmonella* isolates was not possible.

9. Conclusion

This study confirmed that *Shigella* and *Salmonella spp*. are the causative agents of diarrheal diseases. *S .flexneri* was the predominate *spp.* isolated followed by *S. dysenteriae, S .boydii, S. sonnei*. All *Salmonella spp.* were resistant against amoxicillin and Cefaclor whereas one strain of *Shigella* as susceptible to all antibiotics. Most of the *Shigella* and *Salmonella* isolates were resistance against ampicillin, amoxicillin, tetracycline, cotrimoxazole but all are susceptible to norfloxacin and ciprofloxacin. The *Shigella* and *Salmonella* species showed high level of multidrug resistance to the commonly used antibiotics**.**

10. Recommendation

- It is recommended to establish an antimicrobial resistance surveillance system for *Shigella* and *Salmonella species* in different regions of Ethiopia.
- Physicians and clinicians should use ciprofloxacin and norfloxacin as first line antibiotics for the treatment of shigellosis and salmonellosis.
- Avoid overuse of antibiotics to reduce the emergence of multi-drug resistance strains.

11. References

1. Robert F AB. Basic Medical Microbiology. 5th ed. *Little,brown and compny.*1995:345-362.
2. Arias CA, Murray BE. Antibiotic-resistant bugs in the 21st century. A clinical super challenge. *N Eng J Med*. 2009;360(5):439-43.
3. WHO.Weekly Epidemiological report:Shigellosis:Evidence for prevention. A Publication of the Epidemiological Unit, Sri Lanka. 2007; 34, 1-3.
4. Central Statistical Agency Ethiopia and ICF International. Ethiopia Demographic and Health Survey 2011. Addis Ababa, Ethiopia and Calverton, Maryland, USA: *Central Statistical Agency and ICF International.* 2012.
5. Coburn B, Grassl GA, Finlay B. *Salmonella*, the host and disease: a brief review. *Immu Cell biol.* 2006;85(2):112-8.
6. Kumar Y, Sharma A, Sehgal R, Kumar S. Distribution trends of *Salmonella* serovars in India (2001–2005). *Trans Royal Soc Trop Med Hyg.* 2009;103(4):390-4.
7. Huruy K, Kassu A, Mulu A, Gebretsadik S, Andargie G, Tadesse T, Birhan W, Worku N, Ghebreselassie D, Belyhun Y, Yifru S, Adugna S, Tiruneh M. High level of antimicrobial resistance in *Shigella* Species isolated from diarrhoeal patients in University of Gondar teaching Hospital, Gondar, Ethiopia. *Pharmaonline.* 2008;2:328-340
8. Birhaneselassie M, Williams D. A study of *Salmonella* carriage among asymptomatic food-handlers in southern Ethiopia. *Inter J Nutr Food Sci.* 2013; 2(5):243-245.
9. Gu B, Cao Y, Pan S, Zhuang L, Yu R, Peng Z, Qian H, Wei Y, Zhao L, Liu G, Tonq M. Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of *Shigella* between Europe–America and Asia–Africa from 1998 to 2009. *Inter J Antimicro.* 2012;40(1):9-17.
10. Sharma R, Sharma C, Kapoor B. Antibacterial resistance: current problems and possible solutions. *Indian J Med Sci.* 2005;59(3):120.
11. Cheesbrough M. Medical Laboratory Manual for Tropical Countries. Vol. II: Microbiology. Butterworth-Heinemann Ltd, England. 2009:225-431.
12. Huruy K, Kassu A, Mulu A, Worku N, Fetene T, Gebretsadik S, Biadglegne F, Belyhun F, Muche A, Gelaw A, Anagaw A, Yifru S, Wondie Y, Bekele A, Tiruneh M, Reissig D,

Moges F.Intestinal parasitosis and shigellosis among diarrheal patients in Gondar teaching hospital, Northwest Ethiopia. *BMC Res Notes.* 2011;4(1):472.

13. Asrat D. *Shigella a*nd *Salmonella* serogroups and their antibiotic susceptibility patterns in Ethiopia. *East Mediterr Health J.* 2008;14(4):760-7.
14. Beyene G, Asrat D, Mengistu Y, Aseffa A, Wain J. Typhoid fever in Ethiopia. *J Infect Developing Countries.* 2008;2(06):448-53.
15. Braude. A. Antimicrobial drug therapy,Major problems in internal medicine. New York, *WB Saunders.* 1976;8:111-218.
16. Ericsson CD, DuPont HL, Mathewson JJ, West MS, Johnson PC, Bitsura JAM. Treatment of traveler's diarrhea with sulfamethoxazole and trimethoprim and loperamide. *JAMA.* 1990;263(2):257-61.
17. Harnett N. Increasing incidence of resistance among *Shigellae* to trimethoprim. *Lancet.* 1991;337:622.
18. Bauer JD, Ackermann PG, Toro G, Bray WE. Clinical laboratory methods: Mosby; 1974.
19. Temu M, Kaatano G, Miyaye N, Buhalata S, Shushu M, Kishamawe C,Changalucha JM. Antimicrobial susceptibility of *Shigella flexneri* and *S. dysenteriae* isolated from stool specimens of patients with bloody diarrhoea in Mwansa, Tanzania. *Tanza J Health Res.* 2008;9(3):186-9.
20. Orrett FA. Prevalence of *Shigella* serogroups and their antimicrobial resistance patterns in Southern Trinidad. *J Health Pop Nutr.* 2008;26(4):456.
21. Mardaneh J, Poor SA, Afrugh P. Prevalence of *Shigella* species and Antimicrobial Resistance Patterns of Isolated Strains from Infected Pediatrics in Tehran. *Int J Entric Patho.* 2013;1(1).
22. Bassa A, Dadie A, Guessennd N, Gbonon V, Dako E, Dje M, et al. Virulence Factors and Resistance Profile of Shigella Isolated During Infectious Diarrhea in Abidjan, Côte D'Ivoire. *J.Appl. Sci. Rese*., 2010;6(6).594-599
23. Savadkoohi RB, Ahmadpour-Kacho M. Prevalence of *Shigella* species and their antimicrobial resistance patterns at Amirkola children hospital, North of Iran. *Iran J Ped* 2007;17(2).118-122

24. Tiruneh M. Serodiversity and antimicrobial resistance pattern of *Shigella* isolates at Gondar University teaching hospital, Northwest Ethiopia. *Jpn J Infect Dis* 2008;62(2):93-7.
25. Herwana E, Surjawidjaja JE, Salim OC, Indriani N, Bukitwetan P, Lesmana M. *Shigella* associated diarrhea in children in South Jakarta, Indonesia. *Southeast Asian J Trop Med Pub Health.* 2010;41(2):418-25.
26. Brooks JT, Ochieng JB, Kumar L, Okoth G, Shapiro RL, Wells JG. Surveillance for bacterial diarrhea and antimicrobial resistance in rural western Kenya, 1997–2003. *Clinical infectious diseases*. 2006;43(4):393-401.
27. Brooks JT, Shapiro RL, Kumar L, Wells JG, Phillips-Howard PA, Shi Y-P, Vulule JM, Hoekstra RM, Mintze E, Slutsker L. Epidemiology of sporadic bloody diarrhea in rural Western Kenya. *Am J Trop Med Hyg.* 2003;68(6):671-7.
28. Mache A. Antibiotic resistance and sero-groups of *Shigella* among paediatric out-patients in Southern Ethiopia. *E Afr Med* J. 2001;78(6):296-9.
29. Roma B, Worku S, Mariam ST, Langeland N. Antimicrobial susceptibility pattern of *Shigella* isolates in Awassa. *Ethiop J Health Dev*. 2000;14(2):149-54.
30. Getamesay M, Getenet B, Ahmed Z. Prevalence of *Shigella*, *Salmonella* and *Campylobacter* Species and Their Susceptibility Patters Among Under Five Children With Diarrhea in Hawassa Town, South Ethiopia. *Ethiop health sci.* 2014;24(2):101-8.
31. Moges D. Prevalence of *Salmonella* and *Shigella* among Food Handlers in Catering Establishments in Hawassa University, Hawassa, Ethiopia. Master thesis. 2010.16-22
32. Beyene G, Tasew H. Prevalence of intestinal parasite, *Shigella* and *Salmonella* species among diarrheal children in Jimma health center, Jimma southwest Ethiopia: a cross sectional study. *Annals cli micro antim*. 2014;13(1):10.
33. Reda AA, Seyoum B, Yimam J, Andualem G, Fiseha S, Vandeweerd J-M. Antibiotic susceptibility patterns of *Salmonella* and *Shigella* isolates in Harar, Eastern Ethiopia. *J Infect Dis Immun.* 2011;3(8):134-9.
34. Andargie G, Kassu A, Moges F, Tiruneh M, Huruy K. Prevalence of bacteria and intestinal parasites among food-handlers in Gondar town, Northwest Ethiopia. *J Health Pop Nutr* 2008;26(4):451

35. Yismaw O, Negeri C, Kassu A. A five-year antimicrobial resistance pattern observed in *Shigella* species isolated from stool samples in Gondar University Hospital, Northwest Ethiopia. *Ethiop J Health Dev.* 2006;20(3).
36. Andualem B, Kassu A, Diro E, Moges F, Gedefaw M. The prevalence and antimicrobial responses of *Shigella* isolates in HIV-1 infected and uninfected adult diarrhoea patients in north west Ethiopia. *Ethio J Health Dev.* 2007;20(2):99-105
37. Fewtrell L, Kaufmann RB, Kay D, Enanoria W, Haller L, Colford Jr JM. Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *Lancet Infect Dis*. 2005;5(1):42-52.
38. Perilla MJ, Ajello G, Bopp C, Elliott J, Facklam R, Popovic T, Wellys Jet. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. Centers for Disease Control and Prevention, National Center for Infectious Diseases.2003:1-313.
39. He D, Ke B, Deng X, Ke C, Liang Z, Tan H, BS L, MZ L, JD C. Surveillance and analysis on the pathogenic features of *Salmonella* in Guangdong province in 2010. *Chi J Preve Med.* 2012;46(5):424.
40. Diniz-Santos DR, Santana JS, Barretto JR, Andrade MGM, Silva LR. Epidemiological and microbiological aspects of acute bacterial diarrhea in children from Salvador, Bahia, Brazil. *Braz J Infec Dis.* 2005;9(1):77-83.
41. Abdullahi M. Incidence and antimicrobial susceptibility pattern of Salmonella species in children attending some hospitals in kano metropolis, kano state–Nigeria. *Bayero J Pure Appl Sci.* 2010;3(1).
42. Mache A. *Salmonella* serogroups and their antibiotic resistance patterns isolated from diarrhoeal stools of pediatric out-patients in Jimma Hospital and Jimma Health Center, South West Ethiopia. *Ethiop J Health Sci.* 2002;37:37-45.
43. Commission FDRoEPC. Summary and statistical report of the 2007 population and housing census–population size by age and sex. Addis Ababa, December. 2008.
44. Clinical and labratory standard institute. performance standards for antimicrobila suceptibiltiy testing: tweenty third informational supplement. CLSI document M100-S23.clinical and labratory standard institute wvrw, USA.2013.

45. Hamer D, Simon J, Thea D, Keusch G, Hernandez-Avila M, Lazcano-Ponce E. Childhood diarrhea in sub-Saharan Africa. *Inter J Epi.* 1998;27(3):370-6.
46. Ishaku A. Habibu T, Daniel A, Katsa M and and Yakubu H. Antibacterial susceptibilty and Drug resistant pattern of , *Salmonella* sero-groups from Pediatric out-patients at two Health centers in Nasarawa State, Nigeria. *Int. J. Microbiol. Immunol. Res*. 2013; 2(2), 011-016.

12. Annexes

Annex I. Information Sheet and Consent Form

Title of the research project

Prevalence and antimicrobial susceptibility pattern of *Shigella* and *Salmonella* species among diarrheal patients who are attending health institutions in Gondar town, Gondar, North West Ethiopia

Name of the principal investigator: Tesfaye Andualem

Name of the organization: University of Gondar, Gondar college of Medicine and Health Sciences.

Information sheet and consent form prepared for patients who have diarrhea to the study participate in this research project.

Purpose of the research project

The aim of this study is to assess the prevalence and antimicrobial susceptibility pattern of *Shigella* and *Salmonella* species among diarrheal patients who are attending health institutions in Gondar town, North West Ethiopia. The results of this study will be used to design appropriate intervention programs to reduce the incidence of the diseases in Gondar town.

Procedure

The study involves diarrheal patients in Gondar town health institutions. You are selected to be one of the study participants if you are willing to take part in this study and we kindly invite you to take part in our project. If you are willing to participate, we are so happy and we need you to clearly understand the aim of this study and show your agreement. Finally you are kindly requested to give your genuine response in the interview.

Benefits risk and /or Discomfort

By participating in this research project you may feel some discomfort in wasting your time (a maximum of 15 minutes). However, yours participation is definitely necessary for determining the prevalence and antimicrobial susceptibility pattern of *Shigella* and *Salmonella* species among diarrheal patients and to design appropriate strategies and planes to decrease the disease burden .

There is no risk or direct benefit in participating in this research project.

Confidentiality

The information collected from you will be kept confidential and stored in a file, without your name by assigning a code number to it, and no report of the study ever identifies you.

Right to refusal or withdraw

You have the full right to refuse from participating in this research. You have also the full right to withdraw from this study at any time you wish.

Person to contact

This research project will be reviewed and approved by the ethical committee of the University of Gondar. If you have any question you can contact the following individual and you may ask at any time you when want.

Tesfaye Andualem Tele: +251 912877914

E-mail: tesfayeandu@gmail.com

Annex II

የመረጃ ቅፅ በአማርኛ

የትናቱ ረዕስ፡ --የተቅማጥ በሽታን ሊያስከትሉ የሚችሉትን ተዋስያንን መለየትና የፀረ- ተህዋሥያን መድህኒት የመቋቋም ባህሪያቸዉን ማጥናት፡፡

የተመራማሪው ስም፡ ተስፋዮ አንዳአለም

የድርጅቱ ስም፡ በጎንደር ዮንቨረስቲ ህክምና ጠፍ ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ትምህርት ክፍል

የትናቱ አላማ፡ አስፍላጊ የሆኑ የመከላከያ ዘዴዎችን በመንደፍ ህመም እና ሞትን መቀነስ ነዉ፡፡

የትናቱ አተገባበር ፡ ፍቃደገኛ ስለሆኑ እኛ በጣም ደስተኞች ነን፡፡ በሚጨሻም የፍቃደዎን በእርስዎ አስፈላጊ ነው የሚትን መልስ እንዲሰጡ እንጠይቃለን፡፡

ጥቅም አደጋናአለመመቸት፡ - በዚህ ምርምር በመሳተፍው በቀጥታ የሚገኚት ጥቅም ላይኖር ይችላል፡፡ነገረ ግን ወደ 15 ደቂቃ ከስራው ጊዜዎን ሊያባክኑ ይችላሉ፡፡

ሚስጥራዉነት፡ - በዚህ ምርምር ፕሮጀክት የተሰበሰበ መረጃ ሚስጥራዊ ይሆናል፡፡በዚህ ቅፅ የረሰዎ ስም አይፃፍበትም በተጨማሪም እርስዎ ከሚሰጡ መረጃ ጋር አይያያዝም

ያለመሳተፍ ወይም የማቋርጥ መብት፡ በዚህ ጥናት ያለመሳተፍ ፍፀም የሆነ መብት አለወት፡፡

ለተጨማሪ ዝርዝር መረጃ፡ - ከዚህ በታች ባለው አድራሻ ዋና ተመራማሪወን ወይም የትናቱን አማካሪዎች ማግኘት ይቻላሉ፡፡

ተስፍዩ አንዳአለም ሞባይል 2510912877914

- ተስፋዩ አንዳአለም(ተመራማሪ) ሞባይል 0912877914
- ፕሮፌሰር ሞላስ ጥሩነህ(አማካሪ) ሞባይል 0918810357
- ዶክተር ፈለቀ ሞላስ (አማካሪ) ሞባይል 0918776160
- አቶ ዳኛቸው መሉየ (አማካሪ) ሞባይል 0918031335

Annex III: questionnaire

University of Gondar

College of Medicine and Health Sciences

School of Biomedical and Laboratory Sciences

Department of Medical Microbiology

Determining the prevalence and antimicrobial susceptibility patterns of *Shigella* and *Salmonella spp.* among diarrheal patients who are attending health institutions in Gondar town, North West Ethiopia

Written consent

Greeting

Hello!! My name is _____. I am working as data collector in the survey conducted by University of Gondar, College of Medicine and Health Sciences, Department of Medical Microbiology. These questionnaires are prepared to assess the prevalence of *Shigella* and *Salmonella* and theirs antimicrobial susceptibility pattern isolated from stool specimens of diarrheal patients. You are kindly requested to be included in the study, which will have importance in reducing the risk of shigellosis and salmonellosis .The interview will take about 15 minutes and no information concerning you, as an individual will be passed to another individual or institution without your agreement. Your participation is voluntary and you have the right to not participate fully or partially. If you agree to be included in the study I will start my questions by asking general identifications points.

This study is designed to generate information for program expansion and to know the drug of choice for the treatment of the patients who are with shigellosis and salmonellosis and elsewhere

with similar characteristics. To attain this purpose, your honest and genuine participation by responding to the question prepared is very important.

For children assent will be obtained from their parents or guardians.

Confidentiality and consent

We would like to know your some personal issues, your answers and ideas are completely confidential in secured. Your name will not be written on this form. You can refuse to answer a sing question even to the extent to stop the interview at any time you want if you are not comfortable. We appreciate your kindness to be part of the study. The interview will take about 15 minutes. Are you willing to participate?

If the answer yes ☐ ⟶ continue interviewing

No ☐ ⟶ stop interviewing

Section 0: Questioner Identification

01-date------/------/------

02-Questioner identification ----------

Notice: first write or circle the appropriate answer on the coding categories & also

Put on the coding column.

I. Socio demographic characteristics

1. Sex A. Male ☐ B. Female ☐
2. Age (years) _____
3. Occupation. A. government employed☐ B. self employed ☐ C.non employed ☐ D. Merchant ☐ E. Farmer ☐
4. Education. A. Illiterate ☐ B. Only read and Wright☐ C. Primary school (1-8) ☐ D. Senior secondary school (9-12) ☐ E. College diploma and above☐.

5. Residence A Urban ☐ B. Rural☐

II. Clinical profile

1. Did u have abdominal pain? 1.Yes ☐ 2.No☐
2. Did you have headache? 1.Yes ☐ 2.No ☐
3. Did you have vomiting? 1.Yes ☐ 2.No ☐
4. Did you have fever?? 1.Yes ☐ 2.No ☐
5. Did you have joint pain? 1.Yes ☐ 2.No☐
6. Did you have previous diarrhea? A. Yes ☐ B.No ☐
7. If yes for how long it was? A.1-7 day ☐ B.8-13 days ☐ C.14 days and above ☐
8. If yes for questions no 6 what types of diarrhea it was? A. Watery ☐ B.Mucoid ☐ C. Bloody ☐
9. What was its status? A. Stopped ☐ B. Worse ☐ C. Treatment ☐
10. For how long the present diarrhea? A.1-7 day ☐ B.8-13 days ☐ C.14 days and above☐
11. What types of diarrhea it is? A. Watery☐ B. Mucoid☐ C. Bloody☐
12. Where you were ate food from the following places in the last 7- days? A. Home☐ B. Restaurant ☐ C. street vendor ☐ C. another compound ☐
13. Water source A. River ☐ B. Unprotected spring ☐ C. Unprotected well ☐ D. Protected spring☐ E. Stagnant water ☐ F. Protect well ☐ G. Pipe ☐

14. How did you use the water? A. Boiling☐ B. Water guard☐ C. Not boiling ☐

15. Is the health institution accessible? A. Yes ☐ B. No☐

16. What type of toilet do you have in your house? A. Simple Pit latrine ☐ B. open field ☐

17. Do you wash your hands after defecating? A. Yes ☐ B. No☐

18. If yes! A. Regularly with soup ☐ B. Irregularly with soap☐ C. Without soap ☐ D. Not at all ☐

19. Do you wash your hands before preparing food? A. Yes☐ B. No☐

20. If yes! A. Regularly with soup ☐ B. Irregularly with soap☐ C. Without soap ☐ D. Not at all☐

21. Is there solid or liquid waste disposal within the living compound? A.Yes ☐ B.No ☐

22. If yes A. Did you use it ☐ B. Not used ☐

23. If yes for questions no 26, it is nears to yours home? A. Yes ☐ B. No ☐

24. If yes for questions no 26, is it cleaned regularly? A. Yes ☐ B. No ☐

Annex IV; structured Amharic questions

በጎንደር ዩንቨርስቲ ህክምናእና ጤና ሳይንስ ኮሌጅ የህክምና ላብራቶሪ ትምህርት ክፍል

የተቅማጥ በሽታ ጥናት አስሳ

የሚስጥር አጠባበቅ ስምምነት

ጤና ይስጥልኝ አኔ ---------- ባለሁ በጎንደር ዩንቨርሲቲ ህክምና ፋካሊቲ የህክምና ላብራቶሪ ተቋም የጥናት ቡድን አባል ነኝ፡፡

የዚህ ጥናት አላማ በአካባቢው ፡ --- በተቅማጥ ወለድ በሽታዎች የተያዙ ሰዎችን የበሽታ ማጠን ና በሽታወን ሊያጋልጡ የሚችሉ ተዋስያንን በተመለከተ ጥናት ማካሄድ ነው፡፡

ለመሳተፍ ፍቃደኛ ነወት?

1. አዎ----------መልሱ አዎ ከሆነ ወደሚቀጥለው ጥያቄ ዕለፍ/ፊ/
2. የለም----------መልሱ ከሆነ አመሰግነህ/ሽ / ጥያቀቄወን አቻርጭ /ጥ/

መጠይቁን የሞላው ሥም -------መጠይቁ የተደረገበት ቀን----------/------/2006ዓ.ም---

---

በዚህ ጥናት ለመሳትፍ ፍካደኛ ከ ሆኑ ጥያቄወችን በማሳት እጀምራለሁ፡፡

የጥናቱ

ተሳታፊ መለያ ቀጥር----------

1. <u>የሚበራዊ ጥያቄዎችን በተመለከተ</u>

1. ጾታ. A ወንድ ☐ B.ሴት ☐
2. እድሜ(ዓመት) ----------

3. ስራ. A.) የመንግስት ተቀጣሪ ☐ B.) የግል ተቀጣሪ ☐ C.) ስራ የሌለው ☐ D.) ነጋዴ ☐ E.) አረሶአደር ☐

4. የትምህርት ደረጃ. A.) ማንበብእና መጻፍ የሚችል ☐ B.) ማንበብእና መጻፍ የማይችል ☐ C.) 1ኛ ደረጃ(1-8) ☐ D.)2ኛ ደረጃ(9-12) ☐ E.) ዲፕሎማ እና ከዚያ በላይ ☐

5. የመኖሪያ ቦታ A.)ገጠር ☐ B.)ከተማ ☐

2.ከተቅማጥ ህመም ጋር የተያያዙ ጥያቄዎች

1. ሆድ ህመም ይስሞታል? A.) አዎ ☐ B.) የለም ☐
2. ያስታወከዎታል /ያስመልሰዎታል? A.) አዎ ☐ B.) የለም ☐
3. ትኩሳት አለዎት? A.አዎ) ☐ B.) የለም ☐
4. የራስ ህመም ይሰሞታል? A.) አዎ ☐ B.) የለም ☐
5. የመጣጠሚያ ህመም ይሰሞታል?.A.)አዎ ☐ B.) የለም ☐
6. ከዚህ በፊት የተቅማጥ በሽታ ይዞዎት ያውቃል? A.) አዎ ☐ B.) የለም ☐
7. አዎ ከሆነ ለምን ያህልጊዜያት ነበር? A.) 1-7 ቀናት ☐ B.) 8-13ቀናት☐ C.) 14 ቀናት እና ከዚያ በላይ☐
8. ለ6ኛ ጥ ያቄ አዎ ከሆነ ምንአይነት ተቅማጭ ነበረ? A.) ውሃ የመሰለ☐ B.) ንፍጥ መሳይ የተቀላቀለበት ☐ C.) ደም የተቀላቀለበት☐
9. ለ6ኛ ጥ ያቄ አዎ ከሆነ የተቅማጩ ሁኔታ ዕነይት ነበር? A.) ውሂያወኑ የቀመ ☐ B.) በጣም አስከፊ☐ C.) በህክምና የቆመ ☐
10. አሁን በሽታው ከያዝዎት ለምን ያህል ጊዜ ሆነዎት? A.) 1-7 ቀናት☐ B.) 8-13 ቀናት ☐ C.)14 ቀናትእና ከዚያ በላይ☐
11. የአሁኑ ምንአይነት ተቅማጭ ነው ? A.) ውሀየመሰለ ☐ B.) ንፍጥ መሳይ የተቀላቀለበት ☐ C.) ደም የተቀላቀለበት☐
12. ከሚከተሉት ወሰጥ ላለፉት 7 ቀናት የት ነበር የተመላቡት A.) ከቤት የተዘጋጀ☐ B.)ከሆቴል ☐ C.) ከመንገድ ዳር የተዘጋጀ☐ D.)ከነረቢት ቤት ☐

13. ለአገልግሎት የሚጠቀሙበት ውሃ ከየት ነው የሚገኙት? A.) ከወንዝ ☐ B.) ካልተጠበቀ ምንጭ ☐ C.)ካልተጠበቀ ኩሬ ☐ D.)ከተጠበቀ ምንጭ☐ E.) ከተጠበቀ ምንጭ☐ F.)ከቆመ ውሃ ☐ G.)ከቧንቧ ☐ ከጉድጓድ☐

14. .ለማጠጥ የሚጠቀሟት ውሃ እንዴት ነው የሚጠቀሙት A.)ውሃ አጋር ጨምረው ☐ B.)አፍልተው ☐ C.)ሳያፈሉ ☐

15. የጠፍ ተቋም በአካባቢዎ አለ? A.) አዎ ☐ B.)የለም ☐

16. የሚጠቀሙት ሽንትቤት አይነት የትኛው ነው? A.) ከሜዳ ነው የሚዳዱት ☐ B.) የተለምዶ ሽንት ቤት ☐ C.) ሽታ አልባ ሽንት ቤት☐

17. እጅዎን ከማዳጀ ቤት እነደወጡ ይታጠባሉ A .) አዎ☐ B.) የለም ☐

18. አዎ ከሆነ A.)ሁልጊዜ በሳሙና ይታጠባሉ☐ B.)አልፎ አልፎ በሳሙና ይታጠባሉ ☐ C.)ያለሳሙና ይታጠባሉ☐

19. እጅዎን ምግብ ከማዘጋጀተዎ በፊት ይታጠባሉ? A.) አዎ☐ B.) የለም ☐

20. አዎ ከሆነ A.)ሁልጊዜ በሳሙና ይታጠባሉ☐ B.)አልፎ አልፎ በሳሙና ይታጠባሉ ☐ C.)ያለሳሙና ይታጠባሉ☐

21. የፍሳሽ እና የደረቅ ቆሻሻ ማጠየ በአካባቢዎ አለ? A.) አዎ□ B.) የለም □

22. አዎ ከሆነ A.)ይጠቀማሉታል ☐ B.)አይጠቀማሉትም ☐

23. ለ26 ጥያቄ አዎ ካሉ ለማጠቀም ቅርብ ነው A.) አዎ☐ B.) የለም ☐

24. ለ26 ጥያቄ አዎ ካሉ በየጊዜው ይፀዳል A.) አዎ☐ B.) የለም ☐

Annex V Materials used in the study

-MacConkey agar

-*Salmonella Shigella* agar

-Muller Hinton agar

-Triple sugar iron agar

-Selenite F broth

-Simon's citrate agar

-Lysine decarboxylase agar

-Urea agar base

- Motility agar

-Kovac's reagent

-Sterile cotton wool

- Antibiotic disks

- Wire loop

- Sprit lamp

- Petri dish

- Slide, matches

-Diethyl ether

-Antibiotics

Reagent; crystal violate

-Gram's iodine

-Acetone alcohol, safranin

-Cotton, test tube, gauze, forceps

-McFarland standard

-Test tube rack

-Disposable glove, ruler

-Aluminum foil

- Immersion oil

-Microscope

-A balance and weighing papers

- A 1-liter Erlenmeyer flask, cotton plugged or screw capped

- A 1-liter graduated cylinder

-Water proof marker, pen, pencil, paper

-Normal saline ($0.85\%^{w}/_{v}$)

- A 1-liter glass beaker

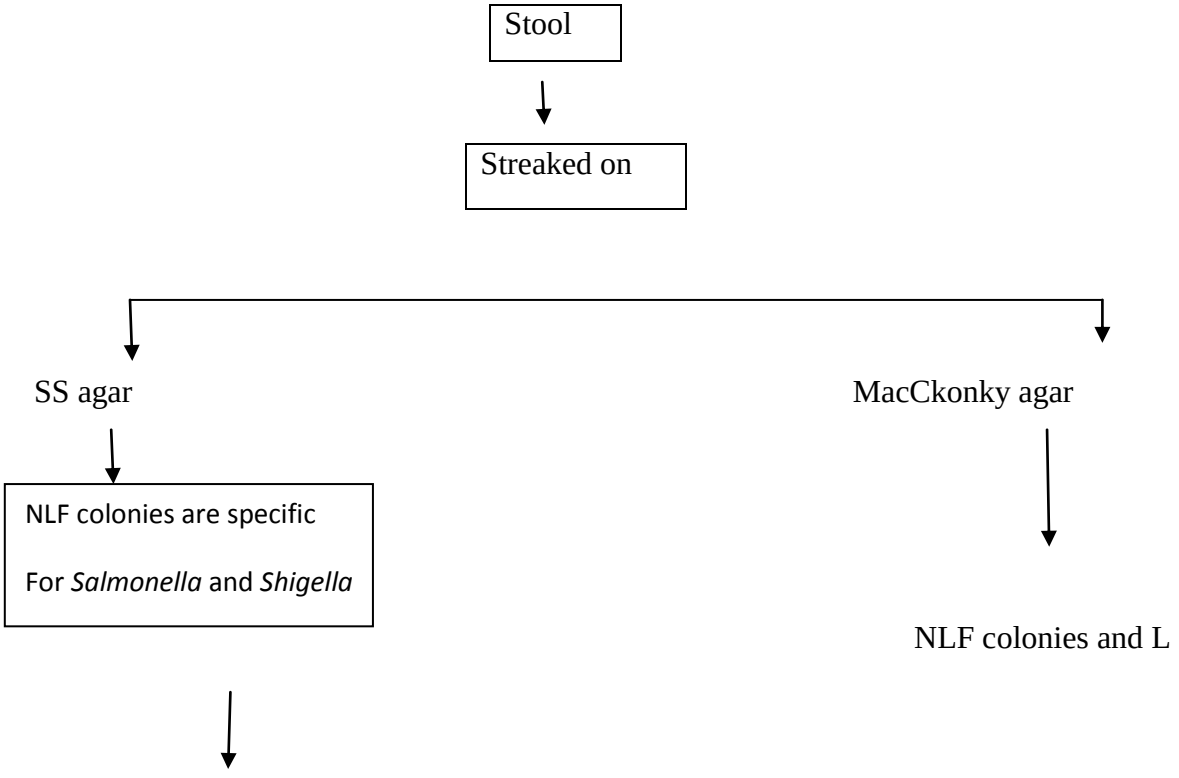
-Distilled water

Annex VI. Sampling selection procedure

The first step will include selection of sources, collection of samples, transportation to the laboratory, isolation and identification of *Shigella* and *Salmonellae* on the basis of their colony morphology, staining property, motility and biochemical and serological characteristics. In the second step, the current statuses of drugs sensitivity and resistance patterns of isolated bacteria will determine.

After collection, each sample will inoculate in to freshly prepare Selenite F broth identified into tube properly and incubated at 370C for 24 hours aerobically in bacteriological incubator. Then, a loop full of bacterial culture from incubated tubes will streak separately into the *Salmonella-Shigella* agar (SSA), McConkey agar (MCA). The plates will examine and study carefully for the presence of characteristic colonies of *Shigella* and *Salmonell*a. The representative *Shigella* and *Salmonella* colonies will characterize morphologically using Gram's staining methods and identifying with biochemical tests

Susceptibility of the isolated *Shigella* and *Salmonella* to different antibacterial agents will perform through disc diffusion method to determine the drug sensitivity pattern.



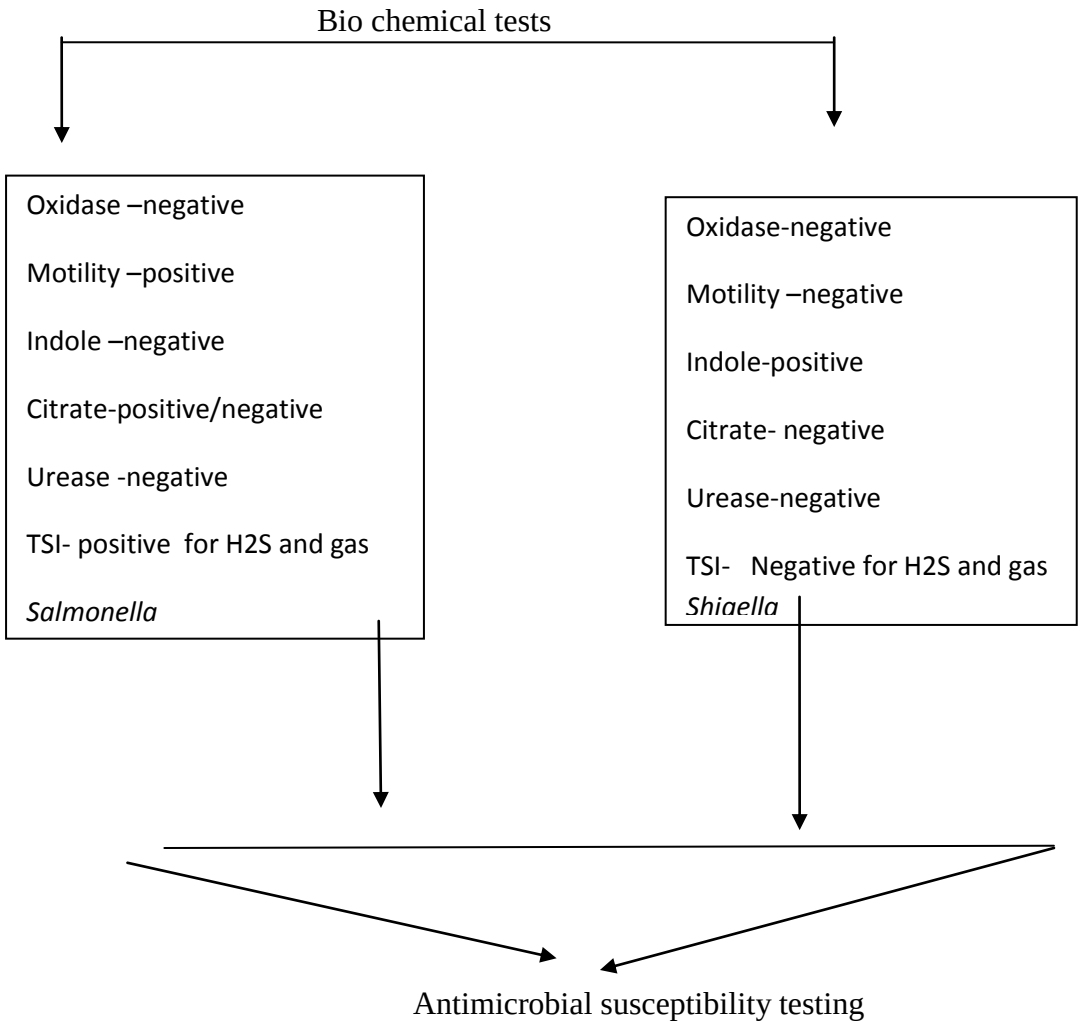


Figure 1

The figure in the above shows that identification of *Shigella and Salmonella* species and their antimicrobial susceptibility pattern.

Antimicrobial susceptibility testing

Procedure

1. Prepare a suspension of the test organism by emulsifying colony of the organism in a small volume of nutrient broth
2. Much the turbidity of suspension with turbidity standard
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).

4. Spread the inoculum evenly over the Muller-Hinton agar plate with the swab
5. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate
6. Incubate the plate aerobically at 35-37^{o}C for 18-24 hours
7. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.
8. Interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard

Declaration

I, the undersigned, Medical Microbiology masters student declare that this thesis is my original work in partial fulfillment of the requirement for the degree of Master of Science in Medical Microbiology.

Name: Tesfaye Andualem

Signature: _____

Place of submission: Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar.

Date of Submission _____

This thesis work has been summated for examination with my /our approval as advisor(s)

Advisors

Name	**Signature**
1. Moges Tiruneh (PhD, Prof.)	----------
2. Feleke Moges (PhD, Assoc. Prof.)	----------
3. Dagnachew Muluye (MSc.)	----------

Examiners'

Name	**Signature**
1.	----------
2.	----------